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## Sample pretreatment by UV photolysis for the ion chromatographic analysis of plant material

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### Abstract

Information on the elemental content of leaves, juices and extracts of vegetables, fruits and plants is of great botanical, nutritional and environmental interest. Procedures are described for the ion chromatographic determination of total chlorine, bromine, phosphorus and sulfur (carbonate–hydrogen carbonate eluent), iron, copper, nickel, zinc and cobalt (pyridine-2,6-dicarboxylic acid eluent) and lead and cadmium ( $\text{H}_2\text{SO}_4$ –HCl–KCl eluent). Detection limits range between 20 and 100  $\mu\text{g/l}$  and the calibration graphs were linear up to 1000–2500  $\mu\text{g/l}$ . In order to dissolve samples prior to chromatographic analysis, oxidative UV photolysis was used; it has decisive advantages over other sample pretreatment methods, owing to the simple procedure and the minimal reagent addition requirement, resulting in minimal contamination. Depending on the type and amount of organic matter, the UV photolysis conditions can be adjusted as required. The homogenized sample is mixed with a small amount of hydrogen peroxide and/or nitric acid and subjected to UV photodegradation in a digester equipped with a high-pressure mercury lamp (500 W); the temperature of the sample was maintained at  $85 \pm 5^\circ\text{C}$  by means of a combined air–water cooling system. Organic constituents are degraded and organic-bound metals and non-metals are set free and their quantification remains unaffected by UV radiation, except for nitrate, iodide and manganese. It follows that chlorine, bromine, phosphorus and sulphur, normally present in different organic compounds, can be quantified as total amount only, without any speciation. The clear solution is injected directly into an ion chromatograph equipped with both a conductivity detector, for the determination of total chloride, bromide, phosphate and sulphate ions, and a postcolumn membrane reactor and variable-wavelength UV–Vis detector, for the determination of lead, cadmium, iron, copper, nickel, zinc and cobalt.

*Keywords:* Plant materials; Sample pretreatment; Inorganic ions

### 1. Introduction

Information on the elemental content of leaves, juices and extracts of vegetables, fruits

and plants is of great botanical, nutritional and environmental interest and so it is important to have reliable methods for their determination. Owing to the strong interfering and/or chelating effect of the organic matrix of these samples, a digestion step with various reagents is usually adopted before any measuring procedure is per-

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formed. The classical methods to destroy organic matter are dry ashing and wet digestion. Dry ashing involves the complete combustion of all the organic matter at high temperature and it suffers from the drawback that the factors controlling the volatilization and retention mechanisms, which lead to low recoveries, are not always easy to assess. Oxidative wet digestion with concentrated acids such as sulphuric, nitric and perchloric acid suffer the disadvantages of high reagent consumption, with consequent significant contamination of the sample, and the long time required. An alternative to these, microwave-based digestion, has become popular owing to the shorter time and lower temperature required for the sample treatment, but the decomposition of organic matter requires strict precautions, and therefore to avoid tedious procedures and sample contamination oxidative UV photolysis was adopted.

The oxidation of organic carbon and the freeing of organic-bound metals and non-metals under influence of UV radiation was initially described by Armstrong et al. [1]. Further investigations [2] into the oxidation of organic materials and the freeing of organic-bound species have indicated that photo-oxidation, in the presence of oxygen or oxidizing agents, is just as efficient as chemical oxidation. In recent years, the decomposition of organic phosphates in water [3] and detergents [4] has been reported.

Most of the botanical materials taken into consideration are completely degraded in less than 2 h, while inorganic constituents, except nitrate, iodide and manganese, have been found to be unaffected by UV radiation. Depending on the type and amount of botanical material to be analysed, the UV photolysis conditions can be adjusted as required.

Numerous analytical techniques have been employed in the determination of trace metals and non-metals in botanical materials, but ion chromatography seems to be one of the most effective and simple to determine both anionic and cationic species owing to its high sensitivity, rapidity, selectivity and simplicity coupled with the advantage of simultaneous determinations.

In this work, a method was developed for the

determination of some microelements in different types of botanical materials such as leaves, juices and extracts of vegetables, fruits and plants: after matrix degradation and the freeing of organic-bound species by UV photolysis, the resulting solution is diluted to a known volume and injected directly into an ion chromatograph. The proposed method has been tested on several types of botanical materials and has been found to be satisfactory for the determination with conductivity detection of total chloride, bromide, phosphate and sulphate (carbonate–hydrogen-carbonate eluent), iron, copper, nickel, zinc and cobalt (pyridine-2,6-dicarboxylic acid eluent) and lead and cadmium ( $\text{H}_2\text{SO}_4\text{–HCl–KCl}$  eluent) [5] with postcolumn spectrophotometric detection.

## 2. Experimental

### 2.1. Reagents and standards

Sodium carbonate, sodium hydrogencarbonate, potassium chloride, 2-dimethylaminoethanol, 4-(2-pyridylazo)resorcinol monosodium salt (PAR) and pyridine-2,6-dicarboxylic acid (PDCA) were of chromatographic grade (Novachimica, Milan, Italy), hydrogen peroxide (30% w/w, without stabilizer), ammonia solution (30%), sodium hydroxide, glacial acetic acid, sulphuric acid (96%), hydrochloric acid (37%) and nitric acid (70%) were Erbatron electronic-grade reagents (Carlo Erba, Milan, Italy). Ammonium acetate solution (2 M, pH 5.5) was of chelation grade (Dionex, Sunnyvale CA, USA). Ultra-pure water with conductivity  $<0.1 \mu\text{S}$  (DI water) was obtained using a Milli-Q four-bowl deionization system (Millipore, Bedford, MA, USA).

Working standard solutions were prepared daily by diluting Carlo Erba Normex atomic absorption standards (1.000 g/l) or by dissolving the required Carlo Erba analytical-reagent grade reagents.

Quartz test-tubes and all glassware were cleaned in concentrated nitric acid and carefully washed with DI water. Normal precautions for trace analysis were observed throughout.

## 2.2. Eluent and postcolumn reagent solutions

A 2.7 mM sodium carbonate–0.3 mM sodium hydrogencarbonate solution was used as the eluent for anions.

For iron(III), copper(II), nickel(II), zinc(II) and cobalt(II) a mixture of 6 mM PDCA, 90 mM acetic acid and 40 mM sodium hydroxide (pH 4.6) and for lead(II) and cadmium(II) a mixture of 75 mM sulphuric acid, 100 mM HCl and 100 mM KCl were used as the eluents [5]. PAR at a concentration of 0.3 mM in 1 M 2-dimethylaminoethanol–0.5 M ammonia–0.5 M sodium hydrogencarbonate was used as the post-column reagent for cationic analysis with both the eluents.

## 2.3. Instrumentation

A laboratory mill (Spex, Edison, NJ, USA), potter and homogenizer (Carlo Erba) of standard performance were used for preparing the samples to be submitted to UV photolysis. The finely ground or homogenized botanical materials were subjected to UV photolysis in a Met-rohm (Herisau, Switzerland) Model 705 UV

digester equipped with a 500-W high-pressure mercury lamp. The temperature of the sample was maintained at  $85 \pm 5^\circ\text{C}$  with the help of a combined air–water cooling system. Full details have already been reported [6].

Chromatographic analyses were performed on a metal-free Dionex DX-300 ion chromatograph equipped with an AGP gradient pump, an IonPac AG12A guard column and an IonPac AS12A anion separator column, an ASRS anion self-regenerating suppressor, an IonPac CG5 guard column and an IonPac CS5 cation separator column, an IonPac CG10 guard column and an IonPac CS10 cation separator column, an IonPac membrane reactor coupled with a postcolumn pneumatic controller for post-column reagent addition, a Dionex CDM conductivity detector and a Dionex DSA UV–VIS multiple-wavelength detector. All the chromatographic conditions are listed in Table 1.

All measurements were made at room temperature and the samples were injected at least in triplicate. All the samples were filtered through a  $0.45\mu\text{m}$  filter prior to injection.

Data collection and the operation of all components in the system were controlled by Dionex AI-450 chromatographic software interfaced via

Table 1  
Ion chromatographic conditions

Parameter	Anions	Fe, Cu, Ni, Zn, Co	Pb, Cd
Column	IonPac AG12A + AS12A	IonPac CG5 + CS5	IonPac CG10 + CS10
Eluent	2.7 mM $\text{Na}_2\text{CO}_3$ – 0.3 mM $\text{NaHCO}_3$	6 mM PDCA– 50 mM $\text{CH}_3\text{COOH}$ – 50 mM $\text{CH}_3\text{COONa}$ (pH 4.6)	75 mM $\text{H}_2\text{SO}_4$ – 100 mM HCl– 100 mM KCl
Eluent flow-rate (ml/min)	1.5	1.0	1.0
Injection volume ( $\mu\text{l}$ )	50	50	50
Detection	Suppressed conductivity	Visible absorbance	Visible absorbance
Suppressor	ASRS	–	–
Controller	Position 2 (100 mA)	–	–
Postcolumn reagent	–	0.3 mM PAR– 1 M 2-dimethylaminoethanol– 0.5 M aq. $\text{NH}_3$ – 0.5 M $\text{NaHCO}_3$	0.3 mM PAR– 1 M 2-dimethylaminoethanol– 0.5 M aq. $\text{NH}_3$ – 0.5 M $\text{NaHCO}_3$
PCR flow-rate (ml/min)	–	0.5	0.5
Detection wavelength (nm)	–	520	520

an ACl-2 advanced computer interface to an 80386-based Epson (Sesto S. Giovanni, Italy) computer.

During the determination of anions, the eluent flow-rate was maintained at 1.5 ml/min, whereas for cations the eluent flow-rate was 1.0 ml/min and that of the postcolumn reagent was 0.5 ml/min; the total flow-rate (1.5 ml/min) was checked at the exit of the waste line. For the best signal-to-noise ratio, the output was measured at 520 nm.

#### 2.4. Sample preparation

A 50–300-mg amount of finely ground or homogenized botanical material was weighted in a quartz tube and 1–2 ml of H<sub>2</sub>O<sub>2</sub> (30%) were added. The quartz tube was closed with a conical PTFE stopper tapered to a point. The stopper acted as cooling finger, prevented solution losses and also protected samples against contamination. The sample was subjected to UV photolysis at 85 ± 5°C for 60–120 min and, after cooling, its volume was made up to 10 ml with DI water and analysed by ion chromatography to determine anions. For the determination of cations, a 20-μl aliquot of concentrated nitric acid was also added during the UV photolysis step in order to ensure the dissolution of any metallic oxide formed during the course of photolysis. When PDCA

eluent was used, as previously specified, 500 μl of 2 M ammonium acetate were added, before making up to volume with DI water, to keep the sample pH into the range 5–6.

### 3. Results and discussion

The detection limits for anions and cations were determined by spiking leaves, juices and vegetables of different composition and origin with various amounts, subjecting them to oxidative UV photolysis for 1–2 h and analysing them by means of the proposed procedure. Detection limits and concentration ranges in which the calibration graphs are linear, with correlation coefficients greater than 0.995, are summarized in Table 2.

The proposed method was applied to the analysis of standard botanical materials with different compositions and origins. Fig. 1 shows the chromatogram for chloride, bromide, phosphorus and sulphur determination (carbonate-hydrogen-carbonate eluent), Fig. 2 that for iron, copper, nickel, zinc and cobalt determination (PDCA eluent) and Fig. 3 that for lead and cadmium (H<sub>2</sub>SO<sub>4</sub>-HCl-KCl eluent), determined in the same sample. The same ions were also determined in different vegetables and botanical materials and, as a rule, the results

Table 2  
Detection limits and linearity ranges in botanical materials after sample pretreatment (see Experimental)

Ion	Eluent	LOD <sup>a</sup> (μg/l)	Linearity range (μg/l)
Cl <sup>-</sup>	Carbonate-hydrogencarbonate	10	20–2000
Br <sup>-</sup>	Carbonate-hydrogencarbonate	20	40–2000
PO <sub>4</sub> <sup>3-</sup>	Carbonate-hydrogencarbonate	25	50–1500
SO <sub>4</sub> <sup>2-</sup>	Carbonate-hydrogencarbonate	25	50–2000
Cd(II)	H <sub>2</sub> SO <sub>4</sub> -HCl-KCl	40	80–1000
Pb(II)	H <sub>2</sub> SO <sub>4</sub> -HCl-KCl	100	200–2000
Fe(II)	PDCA	20	40–2000
Cu(II)	PDCA	20	40–2500
Zn(II)	PDCA	40	80–1500
Ni(II)	PDCA	100	200–2000
Co(II)	PDCA	40	80–2000

<sup>a</sup> Limit of detection: injection loop 50 μl; n = 5; calculated as 3s + average noise.

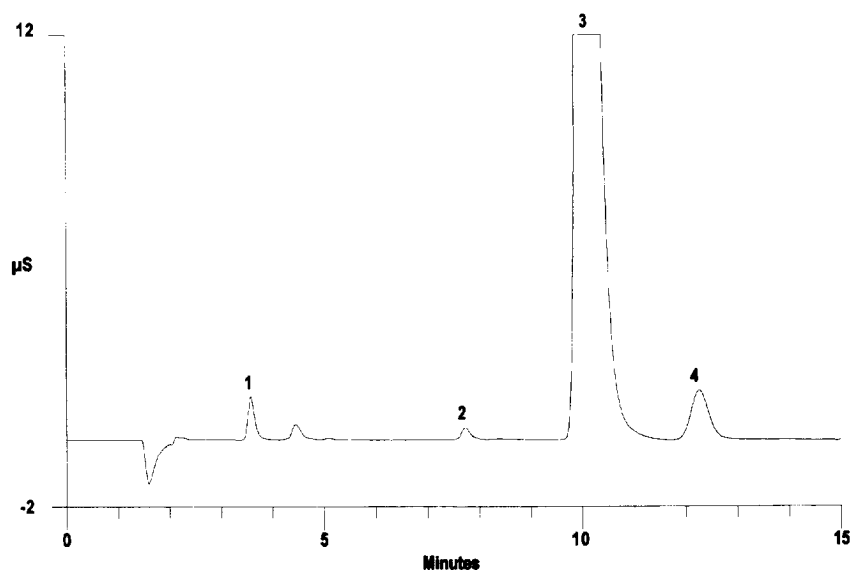


Fig. 1. Chromatogram of anions determined in NIST SRM 1575 Pine Needles using 250 mg of sample prepared as described under Experimental. Chromatographic conditions as in Table 1. Peaks: 1 =  $\text{Cl}^-$  (0.05 mg/l, not certified); 2 =  $\text{Br}^-$  (0.22 mg/l); 3 =  $\text{PO}_4^{3-}$  (93 mg/l); 4 =  $\text{SO}_4^{2-}$  (0.45 mg/l, not certified).

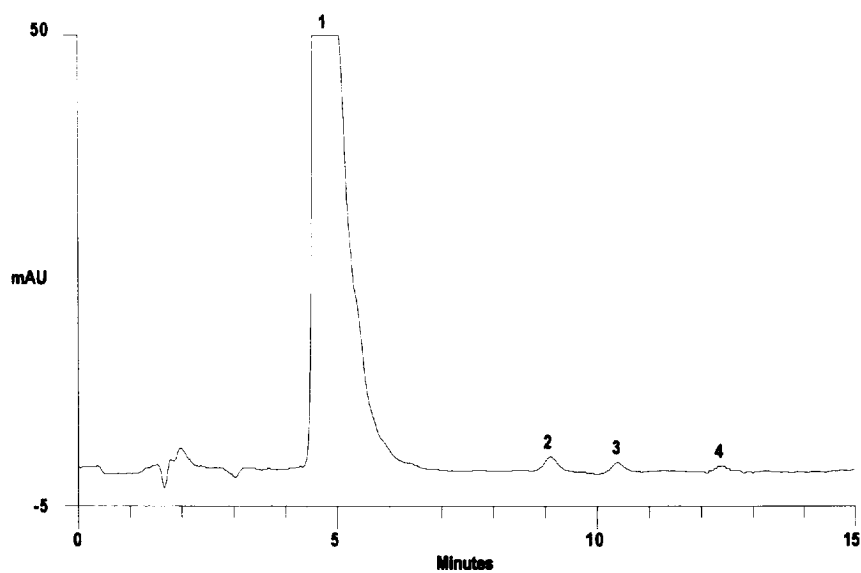


Fig. 2. Chromatogram of metals determined with PDCA eluent in NIST SRM 1575 Pine Needles using 1 g of sample prepared as described under Experimental. Chromatographic conditions as in Table 1. Peaks: 1 =  $\text{Fe}^{3+}$  (20.0 mg/l); 2 =  $\text{Cu}^{2+}$  (0.30 mg/l); 3 =  $\text{Ni}^{2+}$  (0.34 mg/l); 4 =  $\text{Co}^{2+}$  (n.d.).

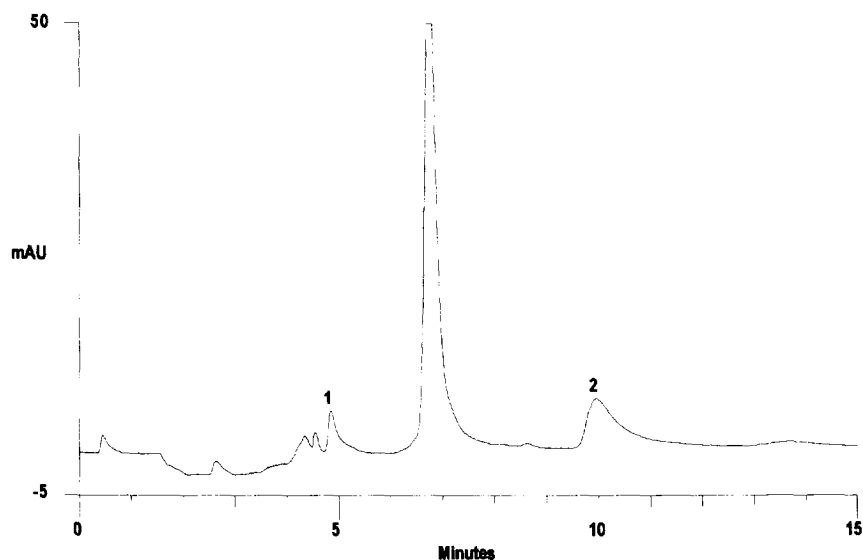


Fig. 3. Chromatogram of metals determined with  $\text{H}_2\text{SO}_4\text{-HCl-KCl}$  eluent in NIST SRM 1575 Pine Needles spiked with 1 mg/l of  $\text{Cd}^{2+}$  and with 1.1 mg/l of  $\text{Pb}^{2+}$ , using 1 g of sample prepared as described under Experimental. Chromatographic conditions as in Table 1. Peaks: 1 =  $\text{Cd}^{2+}$  (1.0 mg/l spike); 2 =  $\text{Pb}^{2+}$  (1.1 mg/l spike).

obtained for any botanical material were strongly affected by its place of origin.

In Table 3 the results obtained on some NIST SRM and LGC standards are reported and are in good agreement with the certified values.

As is evident from Table 2, the proposed method is highly suitable for the determination of low concentrations of iron(III), copper(II),

zinc(II), nickel(II), cobalt(II), lead(II), cadmium(II), chloride, bromide, phosphate and sulphate, but suffers from the drawback that manganese(II), nitrate and iodide are affected by the UV treatment. The detection limits and linearity were found to be in agreement with legal requirements and the elemental concentrations in the samples analysed are generally detectable

Table 3

Comparison of results obtained from the analysis of standard botanical materials

Analyte	NIST SRM 1575 Pine Needles			CRM No. J0-10/c Rice Flour			CRM No. J0-07 Tea Leaves		
	Certified (mg/kg)	Observed (mg/kg)	Recovery (%) <sup>a</sup>	Certified (mg/kg)	Observed (mg/kg)	Recovery (%) <sup>a</sup>	Certified (mg/kg)	Observed (mg/kg)	Recovery (%) <sup>a</sup>
Cl	–	–	–	(230)	232.7 ± 1.1	101.2	–	–	–
Br	(9)	9.2 ± 0.1	102.2	(0.5)	<0.6	–	–	–	–
P-PO <sub>4</sub>	1200	1220 ± 35	101.6	3350	3340 ± 20	99.7	(3700)	3720 ± 25	100.5
Cd	(<0.5)	<1	–	1.82	1.8 ± 0.1	98.9	0.030	<1	–
Pb	–	–	–	–	–	–	0.80	<3	–
Fe	200	200.5 ± 0.8	100.3	11.4	11.6 ± 0.3	101.8	–	–	–
Cu	3.0	3.0 ± 0.1	100.0	4.1	4.0 ± 0.1	97.6	7.0	7.0 ± 0.1	100.0
Zn	–	–	–	23.1	23.4 ± 0.3	101.3	33	32.9 ± 0.5	99.7
Ni	(3.5)	3.4 ± 0.2	97.1	0.3	<3	–	6.5	6.3 ± 0.2	97.0
Co	(0.1)	<1	–	(0.007)	<<1	–	0.12	<1	–

<sup>a</sup> n = 5.

with the dynamic range of the proposed method; in only a few cases, i.e., lead(II) and nickel(II), are the detection limits high in comparison with legal requirements.

An excess of sodium, potassium, calcium, magnesium over 1000:1 was found not to interfere in the determination of both considered anions and cations.

When determining cations with PDCA as eluent, nitric acid is added in order to prevent the formation of any insoluble metal oxide during the course of UV photolysis. If the resulting low-pH solution is analysed directly, the instrumental response is found to be much lower than expected. A systematic study of the response of the considered metals at different pH values revealed that the best pH range is 5–6, otherwise the metals are not completely available for complexation. The buffering effect of adding 500  $\mu$ l of 2 M ammonium acetate solution to the sample was found to be effective.

#### 4. Conclusions

UV photolysis of different botanic materials followed by ion chromatography was found to be

very effective for the simultaneous determination of various inorganic anions and cations. The blank values are particularly low, owing to the minimum amount of reagents required for the sample pretreatment, and the various ions can be determined in the range 20–100  $\mu$ g/l (with calibration graphs that are linear up to 1000–2500  $\mu$ g/l) with excellent resolution and sufficient precision.

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